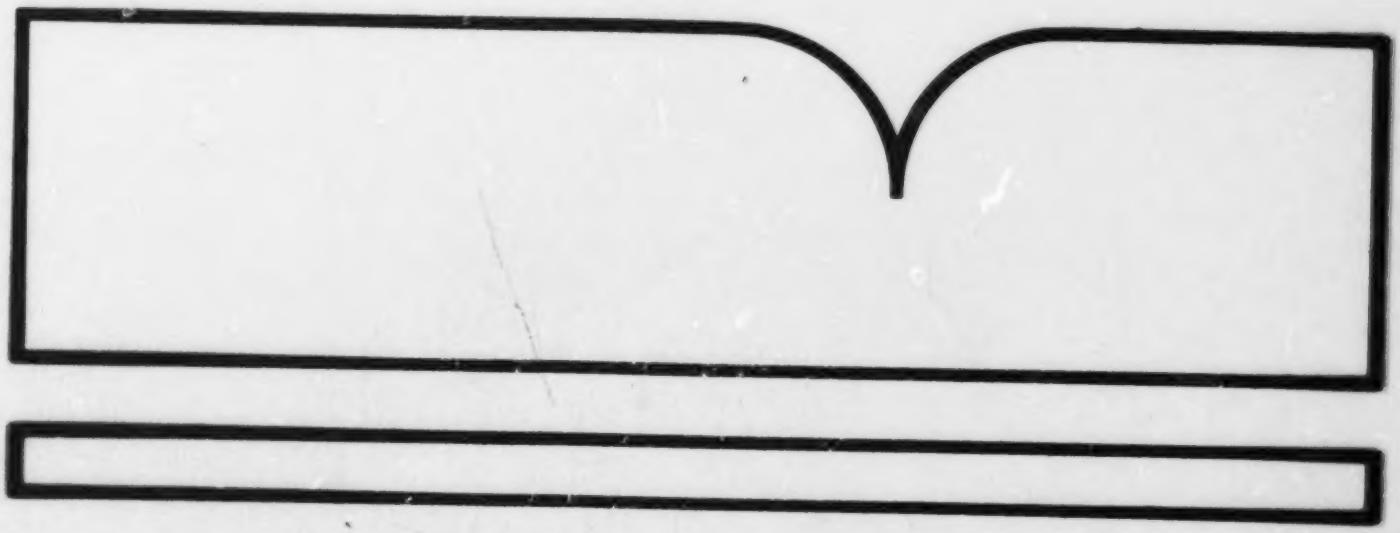
PB85-176634

Uptake Efficiency of the Gills of English Sole (Parophrys vetulus) for Four Phthalate Esters

(U.S.) Corvallis Environmental Research Lab.
Newport, OR

1984



U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/J-84/252	2.
3. RE PB85-176634	
4. TITLE AND SUBTITLE: Uptake Efficiency of the Gills of English Sole (Parophrys velutus) for Four Phthalate Esters (Journal Version)	5. REPORT DATE: 1984
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): B.L. Boese	8. PERFORMING ORGANIZATION REPORT NO.: ERLN-N003
9. PERFORMING ORGANIZATION NAME AND ADDRESS: U.S.EPA, ERLN,Newpore Hatfield Marine Science Center Newport, OR 97365	10. PROGRAM ELEMENT NO.: ACUL1A
	11. CONTRACT/GRANT NO.: NA
12. SPONSORING AGENCY NAME AND ADDRESS: Same as above	13. TYPE OF REPORT AND PERIOD COVERED: Journal Article
	14. SPONSORING AGENCY CODE: EPA600/05

15. SUPPLEMENTARY NOTES: Can. J. Fish. Aquat. Sci. Vol.41, No.11, pp.1713-1718, 1984

16. ABSTRACT

Uptake efficiencies of the gills of English sole (*Parophrys vetulus*) for four homologous phthalic acid esters were directly measured and compared with octanol–water partition coefficients (K_{ow}). Test compounds were diethyl phthalate (DEP), butyl benzyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP), and di-*n*-octyl phthalate (DOC). Mean uptake efficiencies in a single pass through the gills were 11% for DEP (log K_{ow} = 3.2), 42% for BBP (log K_{ow} = 5.8), 3% for DEHP (log K_{ow} = 8.7), and 12% for DOC (log K_{ow} = 9.2). Uptake efficiencies for DEP and BBP were inversely correlated with weight-specific ventilation volume and did not correlate with aqueous concentration. Uptake efficiencies for DOC and DEHP were uncorrelated with any measured parameter. Solubilities of phthalic acid esters were measured by generator column. DEP and BBP had aqueous solubilities similar to published values, but DEHP was 1/700 and DOC was 1/3000 below published solubility values. DOC and DEHP used to determine uptake efficiencies were most likely in colloidal or micellar form, which may be less bioavailable than soluble forms of the compound; this would reduce the apparent uptake efficiency, regardless of K_{ow} value.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT: Distribution Unlimited	19. SECURITY CLASS (This Report): Unclassified	21. NO. OF PAGES: 7
	20. SECURITY CLASS (This page): Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA/600/J-84/252
JOURNAL ARTICLE

NOTES

Uptake Efficiency of the Gills of English Sole (*Parophrys vetulus*) for Four Phthalate Esters

Bruce L. Boese

U.S. Environmental Protection Agency, Marine Division, Corvallis Environmental Research Laboratory, Marine Science Center, Newport, OR 97365, USA

Boese, B. L. 1984. Uptake efficiency of the gills of English sole (*Parophrys vetulus*) for four phthalate esters. Can. J. Fish. Aquat. Sci. 41: 1713–1718.

Uptake efficiencies of the gills of English sole (*Parophrys vetulus*) for four homologous phthalic acid esters were directly measured and compared with octanol–water partition coefficients (K_{ow}). Test compounds were diethyl phthalate (DEP), butyl benzyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP), and di-*n*-octyl phthalate (DOC). Mean uptake efficiencies in a single pass through the gills were 11% for DEP (log K_{ow} = 3.2), 42% for BBP (log K_{ow} = 5.8), 3% for DEHP (log K_{ow} = 8.7), and 12% for DOC (log K_{ow} = 9.2). Uptake efficiencies for DEP and BBP were inversely correlated with weight-specific ventilation volume and did not correlate with aqueous concentration. Uptake efficiencies for DOC and DEHP were uncorrelated with any measured parameter. Solubilities of phthalic acid esters were measured by generator column. DEP and BBP had aqueous solubilities similar to published values, but DEHP was 1/700 and DOC was 1/3000 below published solubility values. DOC and DEHP used to determine uptake efficiencies were most likely in colloidal or micellar form, which may be less bioavailable than soluble forms of the compound; this would reduce the apparent uptake efficiency, regardless of K_{ow} value.

On a mesuré directement l'efficacité de l'absorption de quatre esters phtaliques homologues chez une espèce de sole (*Parophrys vetulus*) et l'on a comparé les résultats aux coefficients de partage octanol–eau (K_{oe}). Les quatres composés étaient : le phtalate de diéthyle (DEP), le phtalate de butyle et de benzyle (BBP), le phtalate de bis(2-éthylhexyle) (DEHP) et le phtalate de di-*n*-octyle (DOC). Les efficacités moyennes d'absorption en un seul passage sur les branchies étaient de 11 % pour le DEP (log K_{oe} = 3,2), 42 % pour le BBP (log K_{oe} = 5,8), 3 % pour le DEHP (log K_{oe} = 8,7) et 12 % pour le DOC (log K_{oe} = 9,2). L'efficacité d'absorption pour le DEP et le BBP était en corrélation inverse avec la ventilation spécifique (v/m) et sans rapport avec la concentration dans l'eau. Pour le DEHP et le DOC il n'y avait corrélation avec aucun des paramètres mesurés. La solubilité des esters phtaliques a été mesurée sur colonne génératrice. Le DEP et le BBP avaient des solubilités dans l'eau conformes aux valeurs publiées, mais le DEHP et le DOC n'avaient, respectivement, que 1/700 et 1/3000 des solubilités publiées. Dans le cas de ces deux derniers esters, nous avions probablement une forme colloïdale ou micellaire moins biodisponibles que les formes solubles ; cela réduirait donc l'efficacité apparente d'absorption, indépendamment de la valeur de K_{oe}.

Received January 27, 1984
Accepted August 8, 1984
(J7667)

Reçu le 27 janvier 1984
Accepté le 8 août 1984

The uptake of numerous organic pollutants from water by fish has been correlated with the compounds' octanol–water partition coefficient (Neely et al. 1974; Lu and Metcalf 1975; Chiou et al. 1977). If pollutant uptake from water is dependent upon lipid and aqueous phase partitioning, then uptake efficiency should be related to the octanol–water partition coefficient (K_{ow}). This hypothesis was indirectly tested (Neely 1979) by deriving the uptake efficiencies of several organic compounds using a mathematical model developed by Norstrom et al. (1976) and regressing these predicted efficiencies against corresponding K_{ow} values. This suggested a linear relationship between uptake efficiencies and K_{ow} over a wide range of K_{ow} values (log 3–12).

The work reported here was designed to test this relationship by directly measuring the concentration of four phthalate esters of differing K_{ow} value in the inspiratory and expiratory flows of English sole (*Parophrys vetulus*). Additionally, the effects of phthalate concentration and ventilatory volume on gill uptake efficiency were measured.

Phthalate esters, used to impart flexibility to plastics, are a homologous group of compounds that vary greatly in K_{ow}. The U.S. Environmental Protection Agency has included phthalate

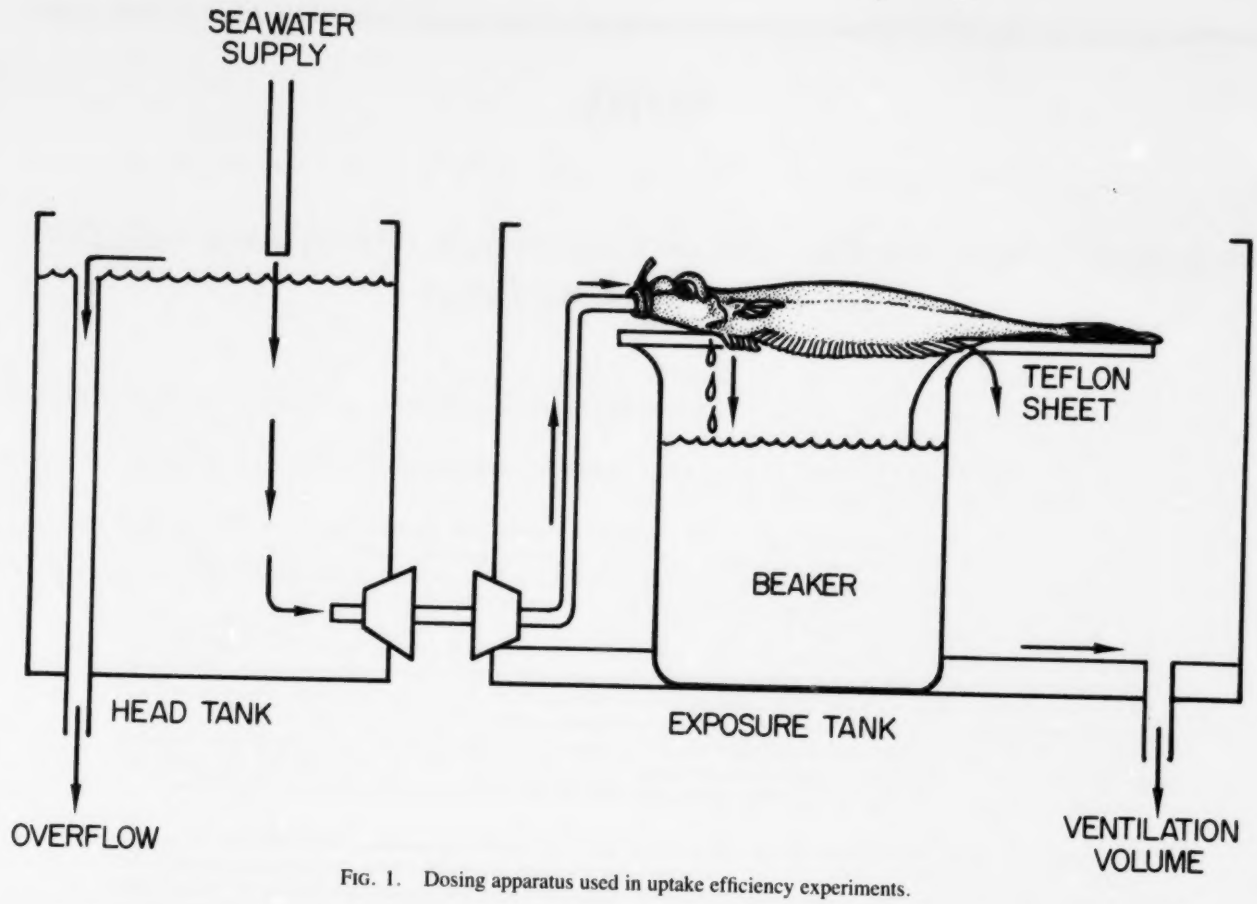


FIG. 1. Dosing apparatus used in uptake efficiency experiments.

esters as priority pollutants because of their high production volumes (Peakall 1975) and their worldwide detection as contaminants in soils, sediments, and animal tissues (Gledhill et al. 1980; Mayer et al. 1972; Ogner and Schnitzer 1970; Morris 1970).

Materials and Methods

Young-of-the-year English sole were collected by otter trawl from Yaquina Bay, OR, and maintained on a diet of Oregon Moist Pellets for at least 3 mo before experimentation. Holding aquaria (250 L) were supplied with aerated seawater (25–32‰, 12 ± 2°C) at a minimum flow rate of $20\,L \cdot h^{-1}$. Total fish biomass never exceeded $2\,g \cdot L^{-1} \cdot d^{-1}$ in any holding aquarium.

Uptake efficiency — The exposure apparatus (Fig. 1) consisted of four rectangular glass aquaria (75 × 200 × 175 mm) connected by Z-shaped glass cannulae (5 mm) to a single glass head tank (300 × 200 × 175 mm). Water level in the head tank was adjusted such that a column of seawater filled the cannula but did not flow out unless pulled by fish respiratory movements.

Fish were anesthetized (0.05% benzocaine) and immobilized by suturing to a 1.6-mm-thick teflon sheet (8 × 15 cm) from which a section had been removed immediately below the opercula. A 250-mL beaker positioned under the opercula served both the support the fish and to collect effluent water.

Glass cannulae in each of the three experimental aquaria were inserted into the mouth of the fish and secured in place with an 8-mm nylon cable tie. Care was taken to minimize injury to the fish and to allow free movement of the floor of the mouth. The body posterior to the gills was covered with moist gauze and the aquarium was covered with aluminum foil to reduce moisture loss. Sand-filtered, UV-sterilized seawater (25–32‰, 12 ± 0.5°C) was supplied to the head tank at $100\,mL \cdot min^{-1}$ for a 2-h recovery period. Water pulled from the head tank by the ventilatory movements of the fish passed through the gills and dripped from the opercula into a collection beaker, with individual fish controlling the ventilation volume. Following the recovery period, seawater in the head tank was replaced by seawater containing dissolved phthalate ester. This dosing solution was prepared by adding an appropriate amount of phthalate stock solution (2–20 mL) to 45 L of sterile seawater (25–32‰, 12°C), followed by vigorous stirring for at least 2 h. This provided a minimum dilution factor for the stock solutions of 1/2000. The phthalate esters tested were butyl benzyl (BBP), di-*n*-octyl (DOC), diethyl (DEP), and bis(2-ethylhezyl) (DEHP) phthalates. Phthalate stock solutions were prepared by dissolving 0.5 g of pure phthalate per litre of acetonitrile carrier. Pure phthalate solutions were obtained from Pfaltz and Bauer, Stamford, CT (BBP), Eastman, Rochester, NY (DEHP), and Chem. Services Inc., West Chester, PA (DOC and DEHP).

Phthalate solutions were supplied to the head tank ($100\,mL \cdot min^{-1}$) via a constant head siphon (Marriotte bottle) for a 3-h exposure. Flow rate of dosing solution was always in excess of that utilized by the fish. In each test, three fish were exposed to a single concentration of phthalate ester. Four to five

TABLE 1. Uptake efficiency exposure conditions and physiological parameters (V/wt = weight-specific ventilation volume).

Phthalate	Concn. range ($\mu g \cdot L^{-1}$)	Mean (SE) fish wt. (g)	V/wt (range) ($L \cdot h^{-1} \cdot g^{-1}$)	Mean (SE) ventilation rate ($beats \cdot min^{-1}$)	n
DEP	30–200	32.7(1.6)	0.009–0.037	32(1)	34
BBP	20–250	28.3(1.0)	0.014–0.065	35(2)	28
DEHP	25–300	26.7(1.3)	0.010–0.060	32(1)	12
DOC	30–40	38.5(2.7)	0.010–0.037	34(2)	13

tests (12–15 fish) were independently performed on DEP, BBP, and DEHP at each of three concentration ranges to test for the effect of phthalate concentration on uptake efficiency. Concentration ranges tested were low (20–35 $\mu g \cdot L^{-1}$), high (190–300 $\mu g \cdot L^{-1}$) and intermediate (95–125 $\mu g \cdot L^{-1}$). For DOC, four replicate tests were made within a single concentration range (30–40 $\mu g \cdot L^{-1}$).

In the control aquarium the cannula height was adjusted so that water dripped into the collection beaker at approximately the same rate as the average ventilation volume of an experimental fish (10–20 $mL \cdot min^{-1}$). Control aquarium water samples were used for inspiratory phthalate determinations. Overflow from the beakers in experimental aquaria was collected to determine ventilation volume. Ventilation rates were determined by counting opercular movements for five 1-min periods at convenient intervals during the experiment. These rates were compared with rates of nonexperimental fish in holding aquaria.

Phthalate analysis — Independent, triplicate, 100-mL samples were taken from expiratory water and control aquaria (inspiratory water) for phthalate concentration measurements beginning 2 h after the addition of phthalate dosing solution. Absolute methanol (10 mL) was added to each sample before phthalate extraction. Phthalate uptake efficiencies were determined by (inspired concn. − expired concn.)/inspired concn. Phthalates were extracted from the water–methanol mixture by alkyl bonded phase columns (C-2 Bond-Elut Analytichem International Inc., Harbor City, CA) following a method used to extract pesticides from water (Andrews and Good 1982). The column retained the phthalates as well as other lipophilic compounds, which were then eluted off with an organic solvent (2 mL acetonitrile). The eluate was analyzed by a Hewlett-Packard 5880 A gas chromatograph (3-μL spitless injections) with a fused silica column (15 m, 0.24 mm ID, liquid-film thickness of 1.0 μm) and flame ionization. The column temperature was elevated in two stages: $30° \cdot min^{-1}$ from 70 to 220°C followed by $15° \cdot min^{-1}$ to 320°C. Recoveries of phthalate esters from control aquaria were 90% (DEP), 72% (BBP), 56% (DEHP), and 63% (DOC) of nominal dose, with a coefficient of variation of 8–15%. These recoveries reflect both analytical error and loss within the system (e.g. adsorption to glass surfaces). Concentrations reported in the text were not corrected for recovery.

Water solubilities — Results of the uptake efficiency experiments brought into question the accuracy of published solubility values for DEHP and DOC. Solubilities of the four phthalate esters were measured using a generator column similar to that described by May (1979). Uncontaminated, filter-sterilized (0.45 μm) seawater was pumped through a sand column containing an excess (1 g phthalate · 100 g $sand^{-1}$) of the compound of interest. The extraction methodology for these samples was changed from that reported above in an attempt to improve recoveries. Phthalates were extracted using C-18 Bond-Elut columns followed by elution with ethylacetate and analyzed by electron capture capillary gas chromatography. Recoveries of phthalate-spiked 100-mL water samples (20–85 $\mu g \cdot L^{-1}$) ranged from 72- for DEHP to 100% for DEP, BBP, and DOC, with a coefficient of variation of 4–8%.

Statistical analysis — Associations between uptake efficiency and physiological–chemical parameters were determined by product-moment correlations at $p < 0.05$ significant level. Regression analysis was by model II regression (Bartlett 1949) using a computer program described by Davies (1971).

Results

Experimental exposure conditions and fish physiology data are summarized in Table 1. Uptake efficiencies for BBP and DEP were inversely correlated with weight-specific ventilation volume ($p < 0.01$) and uncorrelated with fish weight and phthalate exposure concentration ($p > 0.5$). DEHP and DOC uptake efficiencies were not significantly correlated with any measured parameter. Mean ventilation rates did not vary significantly between experiments ($p > 0.5$), nor were experimental ventilation rates significantly different from fish in holding aquaria (mean = 31 $movements \cdot min^{-1}$, $se = 1$, $n = 25$).

As weight-specific ventilation volume increases, uptake efficiency of BBP and DEHP (Fig. 2) decreases. Slopes of the two regression lines are not significantly different ($p > 0.5$) but intercepts and means are different ($p < 0.01$).

Phthalate seawater solubilities (25‰, 12°C) determined by generator column were similar to published values for BBP and DEP, but were 7000 and 3000 times below published values for DEHP and DOC, respectively (Table 2).

Mean uptake efficiency of fish gills increased approximately fourfold as log K_{ow} increased from 3.2 to 5.8 (DEP to BBP) (Table 2). There was no clear relationship between log K_{ow} and uptake efficiencies for DEHP and DOC ($k_{ow} > 8.7$).

Discussion

Uptake efficiency methodology — The dosing apparatus allowed for simultaneous direct measurements of uptake efficiency on three experimental fish with minimal body surface contact with expired water. Ventilation rates of cannulated fish did not differ significantly from uncannulated controls in holding aquaria; however, the wide range of weight-specific ventilation volumes (0.010–0.065 $L \cdot h^{-1} \cdot g^{-1}$) may partially

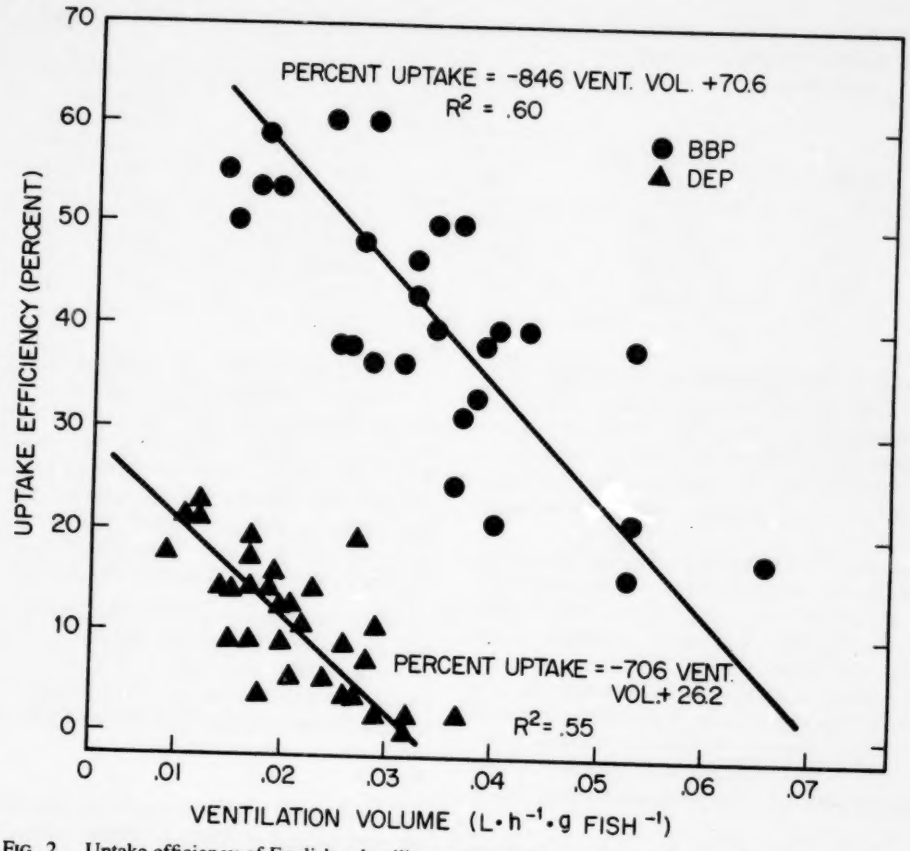


FIG. 2. Uptake efficiency of English sole gills compared with weight-specific ventilation volume. ●, BBP; ▲, DEP.

TABLE 2. Uptake efficiency of English sole gills for four phthalate esters.

Phthalate	log K_{ow}[a]	Solubility ($\mu g \cdot L^{-1}$)[b]	Solubility ($\mu g \cdot L^{-1}$)[a]	Mean uptake (%), $\bar{x}$ (SE)
DEP	3.2	720 000	1 000 000	11.3(1.1)
BBP	5.8	1 900	2 900	42.2(2.5)
DEHP	8.7	0.6	400	2.9(1.2)[c]
DOC	9.2	1.0	3 000	12.4(2.1)[c]

[a]Published solubility values (freshwater at 23–32°C) and K_{ow} values (U.S. Environmental Protection Agency 1980).

[b]Solubility determined in seawater (25‰, 12°C) by the column method described by May (1979).

[c]Uptake efficiencies determined at water concentrations in excess of solubilities determined by generator column.

result from slight hydrostatic head differences between fish. Preliminary experiments showed that fish could control the flux of water through their gills even if the hydrostatic head was elevated or depressed slightly below fish level. Fish could be maintained for up to 7 d in the experimental apparatus; however, abrasion of oral tissue by the cannula limits long-term use.

Elevating fish out of the water was necessary, since the English sole secreted copious amounts of mucous which clogged bonded phase chromatography columns. The presence of mucous did not alter column extraction efficiency; however, the volume of sample that could pass through the column was reduced. Gill mucous may hinder organic uptake, and stress (e.g. chemical, handling) is likely to enhance mucous production. Although gill mucous is a likely source of experimental error, no gross differences in mucous production were noted across the four phthalates tested or among concentrations of any single phthalate.

Effect of ventilation volume — The results showing that DEP and BBP uptake efficiency was inversely correlated to ventilation volume and independent of concentration are consistent with published results for endrin uptake efficiency in brook trout (*Salvelins fortinoulis*) (McKim and Goeden 1982). These reductions in phthalate uptake efficiencies with increasing gill ventilation are likely caused by the same factors that reduce oxygen uptake efficiency at increased ventilation volumes. As gill ventilation increases, diffusion dead space (distance between lamellae) and anatomical dead space (water passing between ends of filaments) increase, and progressively more of the inspired water is shunted away from absorbtive (respiratory) surfaces (Randall 1970). At high ventilation volumes, inspired water may be in contact with the gills for an insufficient time for dissolved oxygen to reach equilibrium with blood (Randall 1970).

Solubility — Large differences were found between published and generator column derived solubility values for DEHP and DOC (Table 2). Although the published values were determined in freshwater and at higher temperatures than used in this study, the disparity between DEHP and DOC solubility

values probably cannot be explained by temperature and salinity differences alone.

Published solubility values for phthalate esters were determined by mixing (shaking or stirring) an excess of phthalate esters with water. For polynuclear aromatic hydrocarbons (PAHs) these mechanical techniques have produced supersaturated solutions with the "dissolved" PAH existing in micellar of colloidal state with water (Peake and Hodgson 1976). Use of generator columns in solubility determinations appears to reduce formation of micelles and colloids (May 1979), yielding lower, and probably more realistic, solubility estimates for compounds with low aqueous solubility.

Effect of k_{ow} — Uptake efficiency of English sole gills was greater for BBP than DEP. This result was expected if uptake efficiency of organic compounds increases with increasing lipid solubility and K_{ow}. If this relationship has general application, an explanation is needed for why DOC and DEHP did not follow the rule. For polychlorinated biphenyls (PCBs), the relationship between bioconcentration factors (BCFs) and K_{ow} also is not linear; with decreasing BCFs noted with log $K_{ow} > 6$ (Sugiura et al. 1978; Shaw and Connell 1982). Suggested explanations for these results are that highly lipophilic PCBs translocate slowly to storage sites within the organism, thus slowing uptake (Sugiura et al. 1978), or that steric effects due to the location and number of chlorines on the PCB molecule hinder uptake (Shaw and Connell 1982). A simpler explanation for the data presented in this paper on the uptake efficiencies for phthalate esters is that exposure concentrations of DOC and DEHP were in excess of their water solubility limits (Table 2), resulting in gills being exposed to micellar or colloidal suspensions of the compounds. This may have reduced uptake efficiencies relative to soluble forms of the same compound due to surface area effects.

In bioconcentration experiments, exceeding solubility limits would extend the time required to reach equilibrium. Mayer (1976), for example, found that as the aqueous concentration of DEHP was increased, the BCF into tissues of fathead minnows (*Pimephales promelas*) decreased. Mayer suggested that this might be the result of increased depuration through induction of detoxification enzymes at the highest exposure concentrations. A more likely explanation is that at the DEHP concentrations used by Mayer (2–62 $\mu g \cdot L^{-1}$), a portion of the compound was not in solution and was not equally available for uptake. Reductions in uptake efficiency with exposure concentrations beyond solubility limits would lengthen the time required for body burden to reach equilibrium, leading to an underestimation of BCF.

When supersaturated solutions are used in bioconcentration experiments, multiphasic uptake might occur due to differential bioavailability of the soluble portion over colloid fractions. Mayer's (1976) data on DEHP uptake into fathead minnows appear to be biphasic with a rapid initial uptake (1–7 d) followed by slower uptake (14–56 d). Since BCF determinations are often made from experiments of limited duration, biphasic uptake could lead to underestimation of BCF.

Implications — In nature, organic compounds exist in soluble, colloidal, and particulate phases. Uptake efficiencies and rates of waterborne organics into tissues of aquatic organisms are likely to be dependent upon these phases. For example, sorption of pesticides to suspended sediments reduces BCFs (Eaton et al. 1983) and acute toxicity (Brungs and Bailey 1967) in fathead minnows. Since compounds in solution probably are taken up more readily through respiratory surfaces than are compounds in suspension, an organism may reach equilibrium more rapidly with the soluble phase than with the colloidal or particulate phase. In some previous bioconcentration studies using hydrophobic compounds, organisms may have been exposed to multiphasic dosing solutions. In these experiments equilibrium body burdens may not have been reached and BCFs were probably underestimated. In short-term experiments, BCFs can only be determined accurately if the exposure concentration of a compound in all its physical–chemical phases is known. Further research is needed to define and quantify these states in nature and to determine how these states affect uptake.

Acknowledgments

I thank Robert Ozretich, Robert Stuart, Dorothy Killian, Fred Roberts, William Schroeder, and Michael Daves for analytical technique development and analysis of samples, Karl Rukavina for fish maintenance, and Steve Ferraro, Henry Lee, Don Baumgartner, and James McKim for critical review of the manuscript. Special thanks to Peggy Gaines for multiple retypings of the manuscript.

References

ANDREWS, J. S., AND T. J. GOOD. 1982. Trace enrichment of pesticides using bonded-phase sorbants. Am. Lab. (Fairfield, Conn.) 11: 70–75.

BARTLETT, M. S. 1949. Fitting a straight line when both variables are subject to error. Biometrics 5: 207–212.

BRUNGS, W. A., AND G. W. BAILEY. 1967. Influence of suspended solids on the acute toxicity of endrin to fathead minnows. Proc. 21st Purdue Indust. Waste Conf., Lafayette, In, Part I, 50: 4–12.

CHIOU, C. T., V. H. FREED, D. W. SCHMEDDLING, AND R. L. KOHNERT. 1977. Partition coefficient and bioaccumulation of selected organic chemicals. Environ. Sci. Technol. 11: 475–478.

DAVIES, R. G. 1971. Computer programming in quantitative biology. Academic Press, New York, NY. 492 p.

EATON, J. G., V. R. MATTSON, L. H. MUELLER, AND D. K. TANNER. 1983. Effects of suspended clay on biconcentration of kelthane in fathead minnows. Arch. Environ. Contam. Toxicol. 12: 439–445.

GLEDHILL, W. E., R. G. KALEY, W. J. ADAMS, O. HICKS, P. R. MICHAEL, AND V. W. SAEGER. 1980. An environmental safety assessment of butyl benzl phthalate. Environ. Sci. Technol. 14: 301–305.

LU, P.-O., AND R. L. METCALF. 1975. Environmental fate and biodegradability of benzene derivatives as studied in a model aquatic ecosystem. Environ. Health Perspect. 10: 269–284.

MAY, W. E. 1979. The solubility behavior of polycylic aromatic hydrocarbons in aqueous systems, p. 142–192. IN L. Petrakis and F. T. Weiss [ed.] Adv. Chem. Ser. No. 185. Petroleum in the marine environment. American Chemical Society, Washington, DC.

MAYER, F. L. 1976. Residue dynamics of di-2-ethylhexyl phthalate in fathead minnows (*Pimephales promelas*). J. Fish. Res. Board Can. 33: 2610–2613.

MAYER, F. L. JR., D. L. STALLING, AND J. L. JOHNSON. 1972. Phthalate esters as environmental contaminants. Nature (Lond.) 238: 411–413.

MCKIM, J. M., AND H. M. GOEDEN. 1982. A direct measure of the uptake efficiency of xeneobiotic chemical across the gills of brook trout (*Salvelinus fontinalis*) under normoxic and hypoxic conditions. Comp. Biochem. Physiol. C, Comp. Pharmacol. 72: 65–74.

MORRIS, R. J. 1970. Phthalic acid in the deep sea jelly fish *Atolla*. Nature (Lond.) 227: 1264.

NEELY, W. B. 1979. Estimating rate constants for the uptake and clearance of chemicals by fish. Environ. Sci. Technol. 13: 1506–1510.

NEELY, W. B., D. R. BRANSON, AND G. E. BLAU. 1974. Partition coefficient to measure bioconcentration potential of organic chemicals in fish. Environ. Health Perspect. 8: 1113–1115.

NORSTROM, R. J., A. E. MCKINNON, AND A. S. W. DEFREITAS. 1976. A bioenergetics-based model for pollutant accumulation by fish. Simulation of PCB and methylmercury residue levels in Ottawa River yellow perch (*Perca flavescens*). J. Fish. Res. Board Can. 33: 248–267.

OGNER, G., AND M. SCHNITZER. 1970. Humic substances: fulvic acid – dialkyl phthalate complexes and their role in pollution. Science (Wash., DC) 170: 317–318.

PEAKALL, D. W. 1975. Phthalate esters: occurrence and biological effects. Residue Rev. 54: 1–41.

PEAKE, H., AND C. J. HODGSON. 1976. Alkanes in aqueous systems. II. The accommodation of C_{12}–C_{36} *N*-alkanes in distilled water. Am. Oil Chem. Soc. 44: 696–702.

RANDALL, D. J. 1970. Gas exchange in fish, p. 253–293. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol. IV. Academic Press, New York, NY.

SHAW, G. R., AND D. W. CONNELL. 1982. Factors influencing concentrations of polychlorinated biphenyls in organisms from an estuarine ecosystem. Aust. Mar. Freshwater Res. 33: 1057–1070.

SUGIURA, K., N. ITO, N. MATSUSMOTO, Y. MIHARA, K. MURATOS, Y. TSUKAKOSHI, AND M. GOTO. 1978. Accumulation of polychlorinated biphenyls and polybrominated biphenyls in fish: limitations of "correlation between partition coefficients and accumulation factors." Chemosphere 7: 731–736.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1980. Ambient water quality criteria for phthalate esters. EPA-440/5-80-067. Washington, DC. p. 22.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.